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Conformational Dynamics of Substrate in the Active Site of Cytochrome P450 BM-3/NPG Complex: Insights from NMR Order Parameters

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Cytochrome P450 enzymes catalyze selective oxidation of a wide variety of hydrophobic substrates.[1] The low-temperature crystal structure P450 BM-3[2] bound to *N*-palmitoylglycine (NPG) shows the substrate bound distant to the iron in a position that is evidently unproductive for chemistry. UV–vis absorbance measurements indicating a spin-state transition and associated studies[3] of cytochrome P450 BM-3 in complex with *N*-palmitoylglycine (NPG) suggest that a conformational change occurs in the active site of the complex where the terminal atoms (which undergo oxidation) of the ligand move from a site distant from the heme iron, as seen in the low-temperature crystal structure to a site proximal to the heme iron at biological temperatures. Prediction of this productive binding mode would be very useful for prediction and control of drug metabolism. We have carried out replica exchange molecular dynamics (REMD) simulations[4] aimed at modeling the structural and dynamic properties of the productive, proximal complex.[5] The population of the proximal state was found to increase with temperature in agreement with UV–vis absorbance and NMR measurements.[3] In addition to conformations characterized by X-ray crystallography and computer modeling, this study[5] showed that a new conformational state, which is stabilized by conformational entropy is significantly populated at room temperature.

Information on fast internal motion of the protein contained in NMR relaxation experiments, can be specified by an effective correlation time and a generalized order parameter S.[6-11] These quantities can be extracted from relaxation data using a fitting procedure. Order parameters[8,12-14] can also be calculated from MD simulations,[15] and here we use REMD trajectories of the P450 BM-3/NPG simulations[5] to calculate S^2 for bond vectors of NPG and compare them to experimentally determined order parameters. This is the first time that NMR order parameters have been calculated from REMD simulations to study the conformational dynamics of a ligand in the active site of a protein.

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Free energy barriers between conformations prevent standard MD sampling often from achieving an equilibrium distribution of bond orientations required for order parameters, obtained from solid-state NMR line shape analysis.[14] REMD[4] is an advanced sampling algorithm that allows crossing of these barriers and facilitates the sampling of a more complete distribution of bond-vector orientations and corresponding order parameters.

We have calculated the order parameters, shown in Figure 1, for the C–C bond vectors of *N*-palmitoylglycine at 294 K from an REMD simulation of P450 BM-3 with 24 replicas and an aggregate simulation time of 72 ns starting from the 1JPZ PDB crystal structure.[5] The value of S^2 for the terminal C–C bond (bond 17) at 294 K is 0.15. This is within the experimental range of 0.12–0.32 (the bar in Figure 1) reported by Jovanovic et al.[14] based on solid-state line-shape analysis. The order parameters were dissected by averaging exclusively over the distal state (green line in Figure 1) and the proximal state (blue line in Figure 1). The value of the calculated order parameter S^2 for bond 17 at 294 K corresponding to the proximal state is within the experimentally determined range, whereas the value of the calculated order parameter corresponding to the distal state is outside the experimentally determined range. The calculated order parameters for the proximal and distal states are consistent with the hypothesis that at room temperature the P450BM3/NPG complex is predominantly in the proximal state.

The order parameters for all C–C bonds for the proximal state are smaller than those for the distal state (compare red and green lines in Figure 1). This implies that the distribution of orientations of bond vectors for the proximal state is wider than that for the distal state. Also the order parameters obtained by averaging exclusively over the proximal state (blue line in Figure 1) is approximately the same as that obtained by complete averaging over both proximal and distal states (red line in Figure 1). This implies that the distribution of orientations of bond vectors corresponding to the distal state overlaps significantly with the distribution of orientations corresponding to the proximal state. If the distribution of bond orientations of distal and proximal states had been very different and the region covered by them did not overlap, the order parameters obtained by averaging over both proximal and distal states, would have been much lower than that of the proximal and of the distal states separately. This is confirmed by the observed distribution of bond orientations for the representative bond 17 shown in Figure 2. The distribution corresponding to the proximal state (blue crosses) covers a significantly larger space as compared to the distal state (green crosses). The wider distribution of orientations of bond vectors corresponding to the proximal state leads to a lower-order parameter for the proximal state as compared to the distal state. Also, because the distribution corresponding to the distal state (green crosses) overlaps with that of the proximal state (blue crosses) and because the population of the proximal state is ~70% at 294 K,[5] the order parameter obtained by averaging over all conformations in the proximal and distal states combined is roughly equal to that obtained by averaging exclusively over the proximal state. We conclude therefore that the low calculated and observed[14] order parameters at room temperature do not primarily reflect conversion between the proximal and distal states, but rather motional averaging within the proximal state.

Measured order parameters are often interpreted in terms of an idealized diffusion in a cone model.[8] Diffusion in a cone of semiangle 60° reproduces the calculated order parameter of 0.15 for bond 17. The distribution of orientations corresponding to the diffusion in a cone model which best represents the distribution of orientations extracted from the simulation is shown in Figure 2 as the gray uniformly shaded area. The contrast between the two distributions, the simulation (blue and green crosses) and diffusion in a cone model (gray shaded region), is evident (see Figure 2). The distribution obtained from the simulation is not uniform. It is much denser at the center of the cone. At the same time, a significant fraction of the points is found outside the cone, with the semiangle as high as 100° from the center. Thus although they result in the same calculated value of S^2, the distribution of orientations of bond vectors obtained from the simulation is qualitatively different from the idealized representation corresponding to the diffusion in a cone.

We have shown earlier[5] that the larger conformational entropy of the proximal state, is responsible for the thermodynamic stability of the proximal state relative to the distal state at room temperature. The wider distribution of bond orientations associated with the proximal state as compared with the distal (see Figure 1) is consistent with this view.[5] The temperature dependence of the order parameter is shown in Figure 3. S^2 for bond 17 decreases with increasing temperature from a value of 0.22 at 264 K to 0.16 at 320 K, in agreement with the trend observed by Jovanovic et al.[14] The observed rate of decrease of S^2 with increasing temperature is mostly due to the increase in population of the proximal state which is characterized by a smaller S^2 than the distal state.[5] If the temperature dependent conformational change were not to occur, the rate of decrease of S^2 with increasing temperature because of thermal motion within the proximal and distal states alone would be predicted to be more gradual than observed. Experimental data on the substrate bound to the reduced form of the enzyme are consistent with a mainly proximal species.[14] It is noteworthy that this species exhibits an order parameter that is within error of that for the oxidized form, despite the fact that the oxidized form involves interchange between proximal and distal sites and the reduced is restricted to the proximal site. The REMD simulations provide an explanation for this observation.

In summary, we have calculated order parameters for ligand motion in the active site of a protein using data extracted from REMD simulations. The calculated order parameter for the terminal bond is within the range extracted from experiment.[14] The rate of decrease of S^2 as a function of temperature is also consistent with the change in populations of the proximal and distal states with increasing temperature. The calculated orientational distribution of the terminal bond differs significantly from that expected based on a simple idealized diffusion in a cone model. We show that the small order parameters for the NPG bond vectors at room temperature are mainly due to the motion within the proximal state rather than transitions from one state to the other.[5] Finally the lower calculated values of the order parameters of the proximal state relative to the distal state is consistent with the proposal[5] for an entropic mechanism as the basis for stabilizing the chemically active (proximal) bound state of NPG to P450 BM-3 at room temperature.

Acknowledgments

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References

1. Montellano, PRO. Cytochrome P450: Structure, Mechanism and Biochemistry. 2. Plenum Press; New York and London: 1995.
2. Haines DC, Tomchick DR, Machius M, Peterson JA. Biochemistry. 2001; 40:13456. [PubMed: 11695892]
3. Jovanovic T, Farid R, Friesner RA, McDermott AE. J Am Chem Soc. 2005; 127:13548. [PubMed: 16190718]
4. Sugita Y, Okamoto Y. Chem Phys Lett. 1999; 314:141.
5. Ravindranathan KP, Gallicchio E, Friesner RA, McDermott AE, Levy RMJ. Am Chem Soc. 2006; 128:5786.
6. Abragam, A. The Principles of Nuclear Magnteism. Oxford University Press; Oxford: 1978.
7. Case DA. Acc Chem Res. 2002; 35:325. [PubMed: 12069616]
8. Liparo G, Szabo A. J Am Chem Soc. 1982; 104:4546.
9. Jarymowycz VA, Stone MJ. Chem ReV. 2006; 106(5):1624. [PubMed: 16683748]
10. Kay LE. J Magn Reson. 2005; 173(2):193. [PubMed: 15780912]
11. Palmer AG, Williams JN, McDermott AE. J Phys Chem. 1996; 100:13293.
12. Levy RM, Karplus M, McCammon JA. J Am Chem Soc. 1981; 103:994.
13. Best RB, Clarke J, Karplus M. J Mol Biol. 2005; 349:185. [PubMed: 15876377]
14. Jovanovic, T., McDermott, AE. 14th International Conference on Cytochromes P450; Dallas, TX. 2005. p. 25
15. Lipari G, Szabo A, Levy RM. Nature. 1982; 300:197.

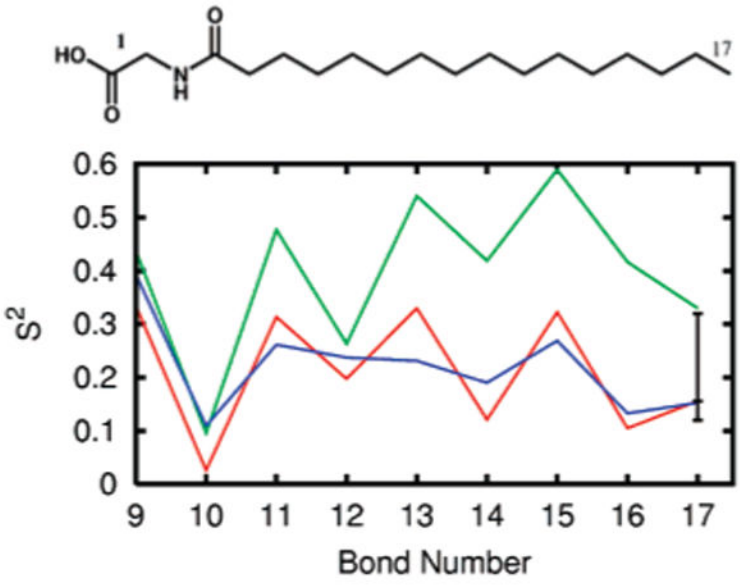

Figure 1.

Order parameters at 294 K as a function of bond index of NPG by averaging over the whole trajectory (red), over exclusively the proximal state (blue), and exclusively the distal state (green). Lower indices correspond to bonds near the carboxylate end of NPG. Index 17 corresponds to the terminal C–C bond farthest from the carboxylate end of NPG and closest to the heme group of the protein. The range of order parameters extracted from experiments[11] at room temperature for bond 17 is represented by the bar on the right (black).

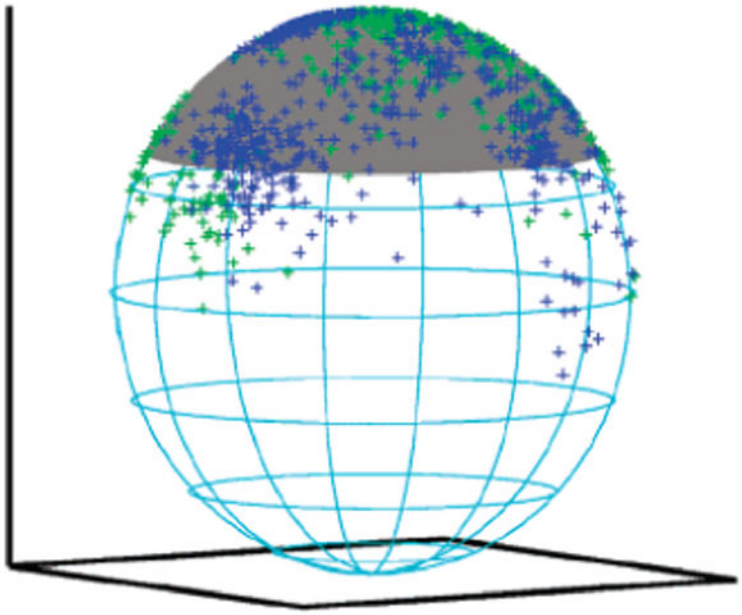

Figure 2.

Distribution of orientations of bond 17 of NPG extracted from the REMD simulation of the P450 BM-3/NPG corresponding to the proximal (blue) and distal (green) states oriented, with the mean orientation pointing along an orientation perpendicular to the plane. The gray shaded area represents the distribution resulting from diffusion in a cone which best represents the distribution of orientations (same S^2 and mean orientation) from the simulation.

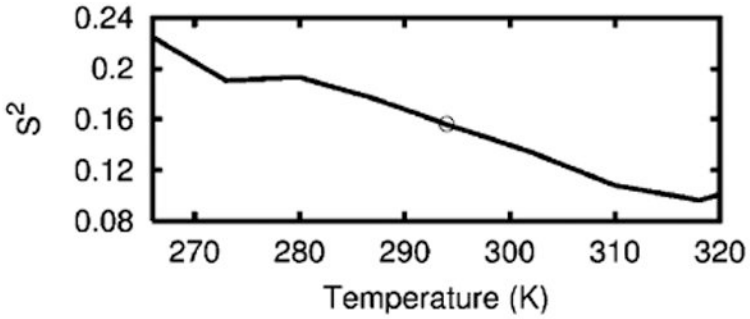

Figure 3.

Order parameter corresponding to the C18–C17 bond vector of NPG (bond 17) extracted from the REMD simulation of the P450 BM-3/NPG complex as a function of temperature. The circle indicates the order parameter at 294 K.